

The University of Chicago

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FEEDING MINERAL OIL TO RATS
FOR PROLONGED PERIODS

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR
OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY
DECEMBER, 1940

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ESTHER DACOSTA

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Introduction

If mineral oil or preparations whose main constituent is mineral oil have any deleterious effects upon nutrition is an important problem, considering their increasing use as laxatives in human therapy. The mineral oil may dissolve and thus remove the fat-soluble vitamins in the food. The increase in intestinal rate induced by the mineral oil may carry the food materials along too rapidly for adequate digestion and absorption. Toxic factors may be present in the mineral oil, injuring the body in other ways. In this paper we report experiments on the utilization of Vitamin A, in butter and cod-liver oil, when mineral oil or Petrol-Agar are fed under different experimental conditions to rats.

Bradley and Gasser¹ recovered both olive oil and petroleum oil from the thoracic duct lymph in dogs fed an emulsion of the two oils, and Channon and Collinson² thought that they had demonstrated mineral oil in the livers of rats and pigs fed the oil by mouth, but the amounts absorbed were too small to be significant as a vehicle for the fat-soluble vitamins.

The experiments of Bloor³ and of Mellanby⁴ showed no demonstrable absorption of mineral oil in dogs and cats. That a depletion of fat-soluble vitamins occurs in animals receiving mineral oil is supported by Burrows and Farr⁵ who fed 14 per cent mineral oil to rats on a balanced diet and found an excess of food fat in the stools and a loss of body weight. When the mineral oil

was fed to rats on an A deficient diet, death resulted in nine days, indicating harmful effects other than the possible vitamin A abstraction. Adding 14 per cent Nujol to a balanced diet, rats died in ten days, and 20 per cent in six days. These rats are reported to have shown signs of A deficiency. Dutcher, Ely and Honeywell⁶ added a mixture of 2.5 of mineral oil and 1 of butter-fat to a vitamin A deficient diet. This mixture contained 67 mgs. of butterfat in seven drops. When seven drops of this mixture was fed separately from the diet deficient in vitamin A the growth impairment and xerophthalmia still occurred; on fourteen and twenty-one drops growth impairment persisted while the rats showed recovery on the above quantities of butter when the mineral oil was not added. Moore,⁷ assaying carotene for vitamin A, reported that if paraffin oil instead of arachis oil was used as a vehicle the availability of vitamin A for rats was decreased. Hawk⁸ et al. report failure to cure experimental rickets in rats by feeding irradiated ergosterol dissolved in mineral oil.

On the other hand, Moness and Christiansen⁹ report that feeding white rats previously deprived of vitamin A mixtures of cod-liver oil and mineral oil and cod-liver oil and olive oil induces equal curative effects. MacDonald, Andes and Briggs¹⁰ state on the basis of colorimetric determinations that the vitamin A content of cream is not reduced by treatment with mineral oil to remove the taste and odor of onion. But Hawks's¹¹ findings render the colorimetric assay of vitamin A questionable. Rowntree¹² fed rats 2-1/2, 5, and 9-1/2 per cent of mineral oil with either butter or cod-liver oil after their being depleted of vitamin A and found no recovery from the vitamin A deficiency when the rats were given the minimal vitamin A requirement. When a sufficient excess of vitamin A (butter, egg-yolk, cod-liver oil) was fed the

mineral oil seemed to have no harmful effects. Jackson¹³ showed that 0.5 cc. of mineral oil divided into three doses per day fed to rats apart from the vitamin A (butter) caused only a slight depression of the growth curve compared with the controls, while when the butter and mineral oil were mixed before being fed a noticeable depression from the normal growth curve resulted.

Olsen¹⁴ studied intestinal motility by the carmine method in three dogs receiving 20 cc. of liquid petrolatum daily and found no appreciable change. There was no decrease in total nitrogen or reducing substances in the feces in these dogs, nor in the six persons receiving 15 cc. of mineral oil per day for a total of 3-1/2 months, alternating monthly with a 2-1/2 non-oil period. No harmful effects were observed, except a slight loss in weight. Lanczos¹⁵ studied cats fluoroscopically and reported that liquid petrolatum hastens the passage of the bismuth meal through the stomach and small intestine. Schlangintweit¹⁶ reports increase in the intestinal rate in mice in proportion to the quantity of oil fed.

Experimental

In our study 237 rats ranging between 40 and 60 gms. at age levels varying from 30 to 40 days were so distributed in the seven experiments that litter mates were always being compared.

Two diets were employed, an adequate one (Diet A), the other deficient in vitamin A (Diet B). Diet A consisted of:

Casein.	18 per cent
Starch.	67 per cent
Inorganic salts (Osborne & Mendel).	4 per cent
Yeast	5 per cent
Butter.	10 per cent

The above diet was fed to all rats at the beginning of the experiment for a control period, to put them in a uniform

good condition. In the deficient diet B the contents were the same except that the casein (Harris') had been so treated that all vitamin A was removed, and Crisco, which is free from vitamin A, was used as the source of fat.

The dosage of mineral oil for the rats was based upon the ratio of the mineral oil prescribed therapeutically for man to his total daily food intake. The average therapeutic dose of mineral oil, i.e., two tablespoonsful per day, weighs approximately 5 per cent of 700 gms., which represents the average daily food intake of man. The rat's daily food intake varies from 10 to 15 gms. Five per cent of the above amounts, which was fed daily to each rat, equalled 0.5 to 0.75 gms. of mineral oil. In a few cases 10 per cent doses of mineral oil (1.0 to 1.5 gms.) were given daily.

In Experiment I, the mineral oil or Petrol-Agar was administered by mouth in the following manner. A hypodermic syringe was fitted with a metal tube ($1/16''$ inside diameter) and by this the mineral oil was introduced into the back part of the mouth and readily swallowed by the rats. In Experiment II, an A deficient diet was fed and mixtures of mineral oil and butter fat were given independently by tube. These mixtures were prepared by mixing weighed quantities of melted butterfat and mineral oil in flasks. The flasks were shaken for 12 hours in a mechanical mixer at approximately 35° C. Then they were stored in the ice box until needed. One mixture (5% butter and 5% mineral oil) consisted of 45 gms. (50 cc.) of melted butter and 45 gms. (56 cc.) of mineral oil. Group III was fed 1.17 to 1.75 cc. of this mixture daily, which furnished .5 to .75 gms. of butter to each rat. This was required as 5 per cent of the rat's daily total food intake. Group IV was fed by tube a mixture (10% butter and 10% mineral

oil) which was prepared the same way as the mixture described above but fed in twice the quantities stated there. Group V was fed a mixture (15% butter and 10% mineral oil) containing 0.515 gms. of butterfat and 0.35 gms. of mineral oil per cubic centimeter. In 2.92 to 4.38 cc. of this mixture each rat was daily furnished with 1.5 to 2.25 gms. of butterfat or 15 per cent of his daily food intake.

In Experiment III, mineral oil or Petrol-Agar was mixed directly with the food.

In Experiment IV, the action of mineral oil per se on butter in vitro was examined by first making a mixture as described above. This mixture was placed in centrifuge tubes, which were stored in the ice box until their contents solidified. After centrifuging them for 30 to 45 minutes, two distinct layers were observed. The upper is referred to later on as the "supernatant" and the bottom solid layer as "recovered" butter. As it was solid at room temperature, before giving it either by tube or mixing it with the food it was melted in an oven at 57° C.

Cod-liver oil as well as butter was used as the source of vitamin A. Although cod-liver oil and mineral oil were perfectly miscible, still mixtures of these were treated as those of butter and mineral oil, i.e., 12 hours in the mechanical mixer at 35° C. When, however, a mixture of Petrol-Agar and cod-liver oil was attempted, the emulsion broke almost as soon as it was removed from the mixer unless a small amount of 1 per cent agar was added before the mixing began. Then a fairly stable emulsion resulted.

Although evidences of vitamin A deficiency (growth and xerophthalmia) were of primary interest, special attention was also given to length of life, the reproductive cycle as evidenced by pregnancies, number and viability of young, etc., and a

complete autopsy at death, paying special attention to the gastrointestinal tract.

In the intestinal rate work which was conducted upon 18 rats (12 females and 6 males), the bead method was used. Thus twenty beads were given orally by means of a tube with a plunger at a definite time daily. The feces were collected at eight-hour intervals, sieved, and the number of beads noted. Different colors were given for six successive days so as to prevent the complication of beginning a color the second time before having recovered all the beads of that color given previously.

Results

Experiment I

In Table I and Graph I are given the major results of Experiment 1. The forty-two rats used for this study have been observed for 695 days.

In Group I, the controls, receiving the adequate diet (diet A), all seven are still alive. They have borne and nurtured twelve litters which have developed and brought up litters of their own.

Group II, receiving diet B, the A deficient diet, died in the following order, two at 50 days, one each at 56, 63 at 68 days, and two at 95 days. They lost weight, averaging 33.2 per cent or 40.2 gms. Forty grams, or 33 per cent, represents the average of the difference in weight between the maximum which this group attained and that at the time of death. This method is followed in all the experiments. Autopsy revealed two cases of pro-stomach ulcers, and xerophthalmia in four of the seven.

In Group III, diet A was given, and in addition 5 per

TABLE I. EXPERIMENT 1.

Group	Diet	Average loss of weight in gms.	Average loss of weight in %	Average length of life		Number of litters	Average number born per litter		Incidence of disease
				Dead	Living		Alive	Dead	
I	Diet A				695+ days for 7	12	7	0	0.0
II	Diet B	-40.2	-33.2	68.1 for 7		0	0	0	3.0
III	Diet A and 5% mineral oil by tube	-50.9	-19.2	293.1 for 6	695+ for 1	3 living 3 dead	4	4	2.7
IV	Diet B and 5% mineral oil by tube	-46.2	-43.4	52.6 for 7		0	0	0	4.0
V	Diet A and 5% Petrol-Agar by tube	-83.4	-29.4	329.2 for 5	695+ for 2	2 living 1 dead	5	4	3.0
VI	Diet B and 5% Petrol-Agar by tube	-26.5	-28.6	50.4 for 7		0	0	0	4.0

Incidence of disease, see page 31.

cent of the weight of the diet in mineral oil by tube daily. The average length of life in this group was 293.1 days. Although an average weight loss of 50.9 gms. occurred, this represented only a 19.2 per cent loss. At autopsy only two revealed gastrointestinal lesions (pro-stomach ulcers). One of these rats (surviving 636 days) had an ovarian tumor about the size of a walnut. But the main cause of death in this group seemed to be associated with reproduction, for the few litters which were born in this group were delivered or nurtured at the expense of the life of the mother. Thus one mother died after delivering four dead foeti. Another ♀ rat was able to bring her litter through weaning, i.e., up to 21 days, and then died. The young lived but were always puny and under-developed. A third ♀ was not able to survive more than ten days lactation. No cases of xerophthalmia developed.

Group IV received diet B and 5 per cent mineral oil by tube. This group lived an average of 52.6 days, dying on the 28th, 30th, 48th, 60th, 63rd, 70th, and 75th day. The average loss of weight of 46.2 gms., or 43.4 per cent, is a little greater than that on the straight A deficiency diet. This occurred although the rats died considerably earlier. This might indicate either a toxic factor in the mineral oil or an interference with the absorption of the food, i.e., by increasing the intestinal rate. No pregnancies occurred in this group. There was a marked increase in gastric lesions in this group compared with the preceding groups. Here five of the seven rats had pro-stomach ulcers. In two cases fissures were also found and in one case even bleeding points or mild erosions were found in the ventriculus. A high incidence of lung infection was also observed, i.e., pneumonia in three cases and tuberculosis in one. It seemed as if the lack of

resistance to infection attributed to A deficiency was increased in this group. Five of the seven had xerophthalmia.

Group V received diet A plus 5 per cent Petrol-Agar by tube. Two ♂s are still alive after 695 days of experimentation. They are not especially active or spry but neither are the controls. The average length of life of the five dead animals was 329.2 days with an age range between 187 to 565 days. They experienced an average weight loss of 83.4 gms., or 29.4 per cent, which is a considerably greater weight loss than from mineral oil. The lesions in the gastro-intestinal tract were negligible except for the ♀ who lived 565 days. This ♀, however, showed many small, round, ulcerated areas in the pro-stomach. Also long, gouged out, tree-like fissures were seen. In the ventriculus clotted blood adhering to ulcerated areas was found and in the antrum besides several very small craters one large ulcer with an active base was seen. This group only gave birth to three litters, one born dead consisting of four members, and two born living having six and four rats respectively. However, before lactation was ended the litter of six was reduced to four. But all four of these reached maturity. Two cases of xerophthalmia developed, one with a severely ulcerated cornea compared with Group III where no xerophthalmia developed in rats receiving mineral oil in the same manner as this group was fed Petrol-Agar.

Group VI was fed 5 per cent Petrol-Agar by tube in addition to diet B. Here the average length of life for the seven animals in the group, all of whom died, was 50 days with an age range between 20 and 86 days. The average weight loss equalled 26.5 gms., or 28.6 per cent, which was considerably lower than for either the straight A deficient animals or the deficient animals being fed mineral oil by tube. Two had pro-stomach ulcers

and three rats showed no gastric lesions. The remaining two had a desquamation of the epithelial lining of the pro-stomach. Two rats showed erosions in the main stomach. Four of the seven had symptoms of xerophthalmia.

Experiment II

In Experiment II an attempt was made to determine whether rats can utilize vitamin A in butter after the butter has been closely mixed with mineral oil for 12 hours at approximately body temperature. Thirty-five rats, divided into five groups of seven each, were fed diet A for a control period of 21 days.

In Group I, receiving diet A, three animals died. The four living animals have been on the experiment 616 days. The three dead animals lived an average length of life of 353 days. They had experienced an average weight loss of 36.0 gms., or 10.6 per cent. Two died from pneumonia. The third showed no gross pathology except that eight foeti were found dead in utero. This group has consistently maintained a higher weight level, as shown in Graph II, than the other groups. Fifteen litters were born with six rats per litter.

Group II was fed the vitamin A free diet, B. All died within 116 days, with an average life span of 102 days. The loss of weight averaged 52.2 gms., or 35.6 per cent, compared with 33.2 per cent loss in the corresponding group of Experiment I. Only three (all of whom were females) showed any lesions in the gastrointestinal tract at necropsy. Two of these had the typical pro-stomach ulcers and one showed a few lesions in the main stomach. One litter of three was born dead. Only three rats had symptoms of xerophthalmia.

TABLE II. EXPERIMENT 2.

Group	Diet	Average loss of weight in gms.	Average loss of weight in %	Average length of life		Number of litters	Average number born per litter		Incidence of disease
				Dead	Living		Alive	Dead	
I	Diet A	-36.0	-10.6	353 for 3	616+ for 4	15	6	0	1.0
II	Diet B	-52.3	-35.6	102.1 for 7		1 dead	3	0	3.3
III	Diet B and mixture of 5% butter and 5% mineral oil by tube	-63.3	-42.6	318.6 for 5	616+ for 2	0	0	0	3.6
IV	Diet B and mixture of 10% butter and 10% mineral oil by tube	-43.5	-38.6	114.2 for 4	616+ for 3	0	0	0	2.9
V	Diet B and mixture of 15% butter and 10% mineral oil by tube	-52.0	-36.3	166.0 for 5	616+ for 2	1 dead 3 living	2	3	2.7

Incidence of disease, see page 31.

Group III, also receiving diet B, was fed by tube the mixture of 5 per cent butter and 5 per cent mineral oil. As the usual daily food intake was 10 gms., this group received 1.17 cc. of a mixture of butterfat and mineral oil which contained 0.5 gm. of butter (0.55 cc.) and 0.5 gm. of mineral oil (0.62 cc.). Thus the rats were being fed 5 per cent of their daily food intake in both butter and mineral oil. Two rats of this group are still living after 616 days of observation. The other five lived an average of 318 days. The first died in 67 days and the last in 616 days. The weight loss averaged 63.3 gms., or 42.6 per cent. The greatest weight loss was shown by the last two who died, i.e., 47.2 per cent and 45.2 per cent. The autopsy findings upon the female who lost 47.2 per cent of her weight showed a pro-stomach studded with small ulcers. The main stomach had streaks of clotted blood and the antrum presented many small craters. In the male there were a few very small ulcers in the antrum. In this rat the xerophthalmia had reached the point of ulceration of the cornea associated with a hemorrhagic exudate. The female had only a mild xerophthalmia. A female who died on the sixty-seventh day showed long fissure-like ulcers and a few round crater ulcers in the pro-stomach, while no gross lesions were found in the male who died on the 181st day. Here the female shows, as in many other cases, an increased severity in gastric findings compared with the male.

Group IV was fed diet B and received daily the mixture of 10 per cent butter and 10 per cent mineral oil by tube. This mixture contained 0.425 gms. of butter and 0.425 gms. of mineral oil in each cubic centimeter. The average daily food intake of this group was 10 gms. Ten per cent of this equalled one gram of butter or 2.34 cc. of the mixture. Rats who had a higher daily food intake were fed in the same manner. Through the whole

experiment these rats have maintained a lower weight level (Graph II) than the controls on diet A while they were receiving the same amount of butter. For 401 days they presented a higher weight curve than Group III, receiving the mixture of 5 per cent butter and 5 per cent mineral oil or the same ratio of the two ingredients but half the quantity. After this time a gradual decline in Group IV was observed. Only three rats are alive. The four dead rats, dying on the 77th, 107th, 129th, and 143rd days, lived an average of 114 days. They never had any litters but there was evidence of three pregnancies in one female. The average weight losses (43.5 gms. or 38.6%) were slightly less in this group than in Group III. The autopsy findings were negative except that one rat presented a marked bilateral patchy alopecia. Xerophthalmia was present in 50 per cent.

Group V received diet B in addition to the mixture fed by tube, containing 15 per cent butterfat and 10 per cent mineral oil. Each rat received 2.34 cc. to 3.5 cc. of this mixture daily. This represented 1.5 gms. to 2.25 gms. of butter and 1.0 to 1.5 gms. of mineral oil. Thus on the basis of a 10 to 15 gram daily food intake per rat, quantities of butter and mineral oil satisfying the requirement of 15 per cent butter and 10 per cent mineral oil were furnished. The mortality rate was higher here than in the other groups, five having died by the 216th day with an average length of life of 166 days. The group presented an average weight level higher than any except the controls. On the 381st day a litter was born. It was dead but that sometimes occurs in first litters in fairly healthy animals. Three other litters followed in close succession. All litters were small, i.e., two rats per litter. They were all poor animals but those in the last litter promise to be normal.

The five dead members of this group showed an average weight loss of 52 gms., or 36.3 per cent. Almost the same weight loss was shown by those on diet B. Four animals developed xerophthalmia. Three rats had gastric lesions: two, pro-stomach ulcers; one, a few slight erosions in the main stomach.

Experiment III

In Experiment III, lasting 525 days, 42 rats were used. These were divided into six groups and placed on the adequate diet for a control period of ten days.

In Group I, receiving diet A, two rats died on the 253rd and 413th days, or lived an average life span of 333 days. The average weight loss was 19.5 gms., or 8.8 per cent. Neither had xerophthalmia but the male showed six well-defined pro-stomach ulcers. This group had maintained a growth curve decidedly higher than the other groups in the experiment.

Group II received diet B. The average length of life in this group was 71 days, the minimum being 56 days and the maximum 80 days. The average weight loss was 30.2 gms., or 20.9 per cent. One litter of four was born dead. At necropsy three cases of xerophthalmia and one case of a female with a few pro-stomach ulcers who had lost 47.5 per cent of her weight were found.

Group III received diet B mixed with 5 per cent of its weight in mineral oil. The seven animals in this group died within 116 days, living an average of 78.6 days. The average weight loss was 31.3 gms., or 33.1 per cent. There were no pregnancies. Five of the seven had xerophthalmia. Pro-stomach ulcers were found in four cases with associated main stomach erosions in one case.

TABLE III. EXPERIMENT 3.

Group	Diet	Average loss of weight in gms.	Average loss of weight in %	Average length of life		Number of litters	Average number born per litter		Incidence of disease
				Dead	Living		Alive	Dead	
I	Diet A	-19.5	-8.8	333 for 2	525* for 5	12	6		0.7
II	Diet B	-30.2	-20.9	71.2 for 7		1 dead	0	4	3.0
III	Diet B and 5% mineral oil in diet	-31.1	-33.1	78.6 for 7		0	0	0	3.8
IV	Diet B and 5% Petrol-Agar in diet	-26.1	-25.5	61.5 for 7		0	0	0	3.5
V	Diet A and 5% mineral oil in diet	-54.0	-27.2	423 for 3	525* for 4	2 dead 5 living	4	3	2.4
VI	Diet A and 5% Petrol-Agar in diet	-49.5	-15.1	406 for 2	525* for 5	2 dead 2 living	3	3	2.4

Incidence of disease, see page 31.

Group IV received diet B and 5 per cent Petrol-Agar mixed with the diet. The life span averaged 61.5 days. The first rat died on the 48th and the last on the 68th day. This was associated with a weight loss of 26.1 gms., or 25.5 per cent, a somewhat greater loss than on straight vitamin A starvation.

Group V received diet A with 5 per cent mineral oil added to the diet. Four of the seven animals in this group are still living. Their weight level as shown in Graph III compares favorably with Groups III and IV of Experiment II when the rats receive vitamin A as butter mixed directly with the mineral oil. In this experiment the mixing is not as intimate but as movement in the stomach mixes the ingredients fed, a fair comparison may be drawn. The fertility in this group differs decidedly from the sterility maintained in the experimental groups in Experiment II. Seven litters were born, two dead and five living. These figures compare best with the rats in Experiment I who were receiving 5 per cent mineral oil by tube. However, the animals reared from the litters born were very poor specimens, i.e., undersized, poor hair conditions, three with an exudate rimming the eyes. The dead members in this group showed an average weight loss of 54 gms., or 27.2 per cent. The greatest weight loss, 39.6 per cent, was experienced by a female. Her stomach showed a few pro-stomach ulcers and the antrum three very small craters. But although the last one to die, a ♂, had no main stomach lesions, the whole pro-stomach was studded with an uneven crust which was pierced by two large ulcers near the ridge. This cornified crust was thickest along the ridge and around the oesophagus at the cardiac end of the stomach. Also, this rat had a severe bilateral patchy alopecia, a profuse, tenacious hemorrhagic exudate from the nose and the lungs when opened revealed many small caseous nodules

indicative of tuberculosis.

Group VI was fed diet A and 5 per cent Petrol-Agar mixed with the diet. The living members (five) have maintained a weight level comparable with the preceding group. They have not been as fertile as Group V, a fact which corresponds with the results on the rats in Experiment I receiving Petrol-Agar by tube who were less fertile than those receiving 5 per cent mineral oil by tube. But the rats surviving from the two litters were better specimens, i.e., heavier, glossier, livelier than the surviving animals from the five litters of the preceding group. Concerning the two who died, both ♀s, a weight loss of 49.5 gms., or 15.1 per cent, was sustained. They had lived 307 and 405 days. The former had no pro-stomach lesions but a small hard white mass about the size of the head of a pin on the main stomach side of the ridge. Also, there were a few small craters in the ventriculus. This rat had ulcerated corneas and a hemorrhagic exudate which formed a crust along the edge of the eyelids and recurred when carefully washed away with warm boric acid solution several times a day. The other female showed one large pro-stomach ulcer and exaggerated rugae in the ventriculus.

Experiment IV

In Experiment IV, 42 rats were studied for 540 days. They were all fed the adequate diet A for an eight-day control period.

In Group I, the adequate diet A was fed. Only four of the seven originally begun have survived. This group delivered nine litters averaging six rats per litter. The three dead members, two females and a male, died on the 152nd, 253rd, and 507th day. No gastro-intestinal lesions were found in any of these

TABLE IV. EXPERIMENT 4.

Group	Diet	Average loss of weight in gms.	Average loss of weight in %	Average length of life		Number of litters	Average number born per litter		Incidence of disease
				Dead	Living		Alive	Dead	
I	Diet A	-13.6	-5.7	274 for 3	540+ for 4	9 living	6		0.5
II	Diet B	-22.8	-24.2	84 for 7		0	0	0	4.1
III	Diet B and 10% recovered butter in diet	-27.0	-14.3	271.5 for 4	540+ for 3	0	0	0	2.3
IV	Diet B and 10% recovered butter by tube	-20.2	-19.2	145 for 6	540+ for 1	0	0	0	2.9
V	Diet A and 5% mineral oil in diet	-55.0	-28.9	276.7 for 4	540+ for 3	3 living	3	0	3.0
VI	Diet B and mixture of 5% mineral oil and 10% butter by tube	-25.6	-20.3	364.6 for 5	540+ for 2	1 dead 1 living	2	0	2.8

Incidence of disease, see page 31.

rats. They showed an average weight loss of 13.6 gms., or 5.7 per cent.

Group II, on diet B, lived an average of 84 days. The first death occurred on the 72nd and the last on the 150th day. The average weight loss was 22.8 gms., or 24.4 per cent. This group was completely sterile, giving no evidence of pregnancies at any time. Upon autopsy all rats showed some gastric pathology. In two cases there were pro-stomach ulcers only and in two others both pro-stomach ulcers and main stomach erosions were found. Xerophthalmia occurred in three of the seven rats.

Group III received diet B plus "recovered butter", the preparation of which was discussed earlier, mixed with the food in 10 per cent quantities comparable with the 10 per cent butter which the controls were fed in diet A. Three of this group still survive and appear to be in excellent condition except that their weight level is decidedly lower than the controls, as shown in Graph III. Those who died did not seem in especially poor condition until near death and then only sustained an average weight loss of 14.3 per cent. Yet in spite of this no live litters have been delivered, although three cases of pregnancy and abortion have been indicated by the typical rise in weight diagnostic of pregnancy and the rapid fall before delivery might occur. The four dead animals lived an average of 271.5 days. The first rat died on the 59th and the last on the 443rd day. The first two who died had neither xerophthalmia nor gastro-intestinal lesions. Of the two rats who died later, one showed a desquamation of the pro-stomach epithelium and the other some erosions in the ventriculus. One rat developed xerophthalmia and a purulent nasal discharge. Six of the rats, dying between the 22nd and 161st days of the experiment, showed severe diarrhea. One of these

rats showed a few fissures in the main stomach. One female rat, the only animal having xerophthalmia in this group, had a few well-defined pro-stomach ulcers and a few fissures and erosions in the ventriculus. The average loss of weight was 20.2 gms., or 19.2 per cent. This group never showed any signs of pregnancy. Two females of tried fertility were placed with the lone surviving male for months and showed no signs of pregnancy.

Group V was fed diet A with 5 per cent mineral oil added and mixed with it before ingestion. Only three of the rats begun are alive. These rats, as the rats in Groups V and VI of Experiment III who are on a similar regimen, have an oily appearance due to "leakage" of the oil at defecation. The living rats have maintained a higher weight level than either of the preceding groups. But they have maintained a lower weight level (Graph IV) than in a comparable group (Group V) in Experiment III (Graph III). Three litters have been born and reared. They were very small litters, numbering 2, 3, and 4. The dead members lived an average of 277 days. The average weight loss was 55 gms., or 28.9 per cent. There was a 50 per cent occurrence of xerophthalmia and a 75 per cent of gastric lesions in the four who died. In one case two slight erosions of the ventriculus were found but in those who lived longer, one had a few pin-point craters in the antrum and the other two large well-defined pro-stomach ulcers with a few erosions in the ventriculus.

Of the seven rats in Group VI only two are alive. They received diet B and the mixture of 10 per cent butter and 5 per cent mineral oil by tube. This mixture was fed in 1.76 cc. to 2.64 cc. doses daily to each rat. This furnished 1 to 1.5 gms. of butter and 0.5 to 0.75 gms. of mineral oil, which satisfies the requirement stated above that 10 per cent of the diet as

butter and 5 per cent of the diet as mineral oil is being fed in the mixture daily to each rat. The survivors are in fairly good condition as indicated by the growth curve (Graph IV). Four litters were delivered by this group but only two survived and one gives promise of reaching adulthood. The average weight loss was 26.5 gms., or 20.3 per cent. Two of the rats developed xerophthalmia.

Experiment V

In Experiment V cod-liver oil was used as the source of vitamin A as well as butter. Seven animals were used per group, or 28 for the experiment. The first group received a modified diet A, instead of 10 per cent butter, a mixture of 9 per cent butter and 1 per cent cod-liver oil as the vitamin A content of the diet. Three of these animals, all ♀s, died within 173 days, i.e., one on the 167th and two on the 198th days. Their average weight loss was 9 gms., or 7.9 per cent. The four who have been on the experiment for 487 days have maintained a consistently higher weight than the other groups as shown in Table V. Due to their being only one surviving sex, pregnancies were impossible after the three ♀s had died, but each had had one pregnancy and successful delivery before dying. Also, three different females have been placed with these males and all have been rendered pregnant.

Group II, on diet B, are all dead, but due to the fact that they were given a long control period (38 days) on diet A before beginning the deficient diet B, their storage of vitamin A was such that they lived an average of 186.1 days, the maximum length of life being 237 and the minimum 156 days. The average

TABLE V. EXPERIMENT 5.

Group	Diet	Average loss of weight in gms.	Average loss of weight in %	Average length of life		Number of litters	Average number born per litter		Incidence of disease
				Dead	Living		Alive	Dead	
I	Modified diet A and 1% cod liver oil	-9.0	-7.9	71 for 3	487+ for 5	3	7	0	0.5
II	Diet B	-60.3	-34.4	186.1 for 7		2 dead	2	3	4.1
III	Modified diet A and mixture of 1% cod liver oil and 5% mineral oil by tube	-32.0	-31.7	192.6 for 3	487+ for 4	2 dead 1 living	1	3	2.5
IV	Modified diet A and mixture of 2% cod liver oil and 5% mineral oil by tube	-41.7	-27.4	257.2 for 3	487+ for 3	1 dead 4 living	2	1	2.7

Incidence of disease, see page 31.

weight loss, as shown in Table V, was 60.3 gms., or 34.4 per cent. These heavy weight losses are comparable with those found in Experiments I and II rather than the lesser weight losses in Experiments III and IV. This group gave birth to two litters, one born dead and the other, a litter of two, were supported through lactation and weaning but died on the 35th and 38th days. At necropsy four had xerophthalmia. In only one case, a ♀, was this associated with corneal ulceration. The gastro-intestinal findings were negative except in two cases, one where a few main stomach erosions and the other where multiple round ulcers in the pro-stomach, streaks of clotted blood in the ventriculus as well as a few minute lesions in the antrum were found. One ♂ had a very large cyst in the left kidney but this was not associated with any other macroscopic pathology.

Group III received the modified diet A and the mixture of 5 per cent mineral oil and 1 per cent cod-liver oil by tube. This mixture was fed in 0.825 cc. to 1.237 cc. doses which contained 0.1 to 0.15 gm. of cod-liver oil and 0.5 gm. to 0.75 gm. of mineral oil. These quantities represented 1 per cent by weight of the daily food intake in cod-liver oil and 5 per cent by weight of the daily food intake of the rat in mineral oil as the rat consumes from 10 to 15 gms. daily. The four surviving animals are in excellent physical condition, i.e., their hair coats being sleek and silky. But their body weights are markedly lower than that of the controls. Also, eye conditions are not as healthy as in the controls, for one female has lost hair around the eyes and the lids are swollen and reddened while a hemorrhagic exudate which can be washed away returns within 24 hours. No ulceration or clouding of the cornea has occurred yet.

Of the three dead animals in this group, two ♀s and a ♂,

two had xerophthalmia. All had some gastro-intestinal pathology. One ♀ had a large, well-defined pro-stomach ulcer while the other ♀ had long streaks of clotted blood and several erosions in the main stomach. The ♂ had a few small pro-stomach ulcers. The length of life for these three rats was 192.6 days. A loss of weight of 32 gms., or 31.7 per cent, was observed. These figures are similar to those for those on straight vitamin A deficiency (diet B). Only three litters were born and two of these were dead. The one animal in the surviving litter only lived through lactation and then died. However, evidence of death and resorption of fetuses has been indicated four times, a fact which was not noticed in the straight vitamin A starvation group.

Group IV received modified diet A and a mixture of 2 per cent cod-liver oil and 5 per cent mineral oil by tube. This mixture was fed in 1.02 to 1.53 cc. doses daily to each rat depending upon whether the total daily food intake was 10 or 15 grams. In this way each rat was fed by tube daily 0.2 to 0.3 gms. of cod-liver oil and 0.5 to 0.75 cc. of mineral oil, which represents 2 per cent of the total daily food intake in cod-liver oil and 5 per cent of the daily food intake in mineral oil. Of the seven animals begun on this experiment, only three have survived who have maintained a weight throughout consistently higher than Group III on a similar regimen except receiving 1 per cent cod-liver oil and lower than Group I on diet A and 1 per cent cod-liver oil. They have born five litters, four born living, but only two of these have reached adulthood and thus far have proved sterile. Of the four who died two were ♂s and two ♀s, the average length of life being 257 days with a minimum of 100 and a maximum of 456 days. Three of the rats developed xerophthalmia, in one case associated with a hemorrhagic exudate.

TABLE VI. EXPERIMENT 6.

Group	Diet	Average loss of weight in gms.	Average loss of weight in %	Average length of life		Number of litters	Average number born per litter		Incidence of disease
				Dead	Living		Alive	Dead	
I	Diet A				475+ for 5	6 living	7		0.0
II	Diet B	-67.0	-31.8	176.6 for 5		1 dead	4	0	3.8
III	Diet B and mixture of 10% butter and 5% mineral oil by tube	-33.6	-27.8	389 for 2	475+ for 3	1 dead 1 living	3	2	2.4
IV	Diet B and 10% supernatant by tube	-55.0	-26.5	339.5 for 2	475+ for 3	0	0	0	2.8
V	Diet B and 10% recovered butter by tube	-31.3	-37.5	220 for 3	475+ for 2	0	0	0	2.8
VI	Diet B and mixture of 10% butter (vitamin A destroyed) and 5% mineral oil by tube	-40.6	-27.8	161 for 5		0	0	0	5.0

Incidence of disease, see page 31.

Experiment VI

In Experiment VI, 25 animals were used, 5 per group. They were all fed diet A for a control period of 35 days. The experiment has been conducted for 475 days and the results in Table VI and Graph VI were obtained. Group I received diet A. The six litters born in this group averaged seven per litter and developed into normal rats.

Group II was put on diet B. These animals lived an average of 176 days, the first dying on the 123rd and the last on the 274th day. The average loss of weight was 67 gms., or 31.8 per cent. In this group, one litter of four was born.

Group III received diet B and a mixture of 10 per cent butter and 5 per cent mineral oil by tube. These rats are maintaining a weight level somewhat lower than the controls. The two animals who died had lived 356 and 422 days respectively or an average of 389 days. They showed a weight loss of 38.6 gms. or 27.8 per cent. Although the living animals are in good physical condition, those who died were extremely emaciated. The ♀ had a large abscess in the left lung and an enlarged cyanotic oviduct. In both animals the pro-stomach was free from lesions but in the ♂ long streaks of clotted blood were found in the main stomach and a few shallow lesions in the antrum.

Group IV received diet B and by tube 1.76 to 2.64 cc. of "supernatant" which furnished approximately 1 gm. to 1.5 gms. of butter and 0.5 to 0.75 gms. of mineral oil. In this way each rat was being fed daily approximately 10 per cent of the diet by weight in butter and 5 per cent in mineral oil. This mixture is

even more thorough than that prepared for the preceding group, for the butter and mineral oil do not separate after 30 to 45 minutes centrifuging. The vitamin A seems slightly less available under these conditions, for although there are the same number of living animals in both groups, i.e., two, still in this group with one exception the weight has been slightly lower throughout. Then the length of life for the dead rats was slightly less, i.e., 339 days for the average compared with 389. Weight loss averaged 55 gms., or 26.5 per cent, compared to 27.8 per cent for Group III. This group had no litters. Twice there was evidence of abortion but no deliveries occurred. While Group III had two litters, one born dead and the living litter of three survived for a time but died on the 16th, 30th, and 31st days.

Group V received diet B and 10 per cent of the "recovered" butter by tube. Only two members are still living and although they weigh less than all preceding groups, they are not suffering from eye troubles, loss of hair, or diarrhea. The average length of life of those who died in this group, 253 days, is considerably less than in the two preceding groups. But the weight loss, although greater in grams, i.e., 55, than Groups III and IV, is about the same in per cent, i.e., 26.5. This would indicate that the growth was better in this group than the others. There were no pregnancies in this group as in rats treated the same way in Experiment IV. The rat dying on the 137th day showed pneumonia but no gastric lesions. The animals who lived longer had more interesting lesions. The ♂ dying on the 271st day had ulcers and shallow fissures in the pro-stomach. The ♀ who died on the 352nd day had several pro-stomach ulcers and mild erosions in the ventriculus.

Group V was fed diet B and the mixture of 10 per cent

butter with the vitamin A destroyed by heat and 5 per cent mineral oil by tube. The vitamin A was destroyed by placing the butterfat in an open vessel in an oven at 102° C. for three days and then mixing it with mineral oil. The average length of life was 161 days with the minimum at 51 days and the maximum at 207. The average loss of weight of 40.6 gms., or 27.8 per cent is in line with the weight losses in all preceding groups except the controls. This group never had any indications of pregnancies, although several rats lived long enough to rear several families. The autopsy findings were more serious in this group than in any other including those on straight A deficiency. Four of the five had xerophthalmia. Two of these were the severe ulcerative type with a hemorrhagic exudate. Three rats showed that oily-haired appearance referred to above. Only one had definite lesions, i.e., fissures in the pro-stomach and two small craters in the antrum, while a second rat had a few mild erosions in the ventriculus.

Experiment VII

Experiment VII represents the results on eighteen rats, three per group. The experiment has run for 415 days. All rats in this experiment were fed diet A for a 35 day control period.

Group I received diet A. Their fertility was evidenced by ten litters averaging seven per litter, born and reared and reached normal adulthood in a healthy, well-nurtured condition. This group is still living.

Group II, receiving diet B, showed a weight loss of 51.3 gms., or 30.4 per cent, which is similar to the weight losses in other straight A deficiency groups. Their average length of life

TABLE VII. EXPERIMENT 7.

Group	Diet	Average loss of weight in gms.	Average weight in %	Average length of life		Number of litters	Average number born per litter		Incidence of disease
				Dead	Living		Alive	Dead	
I	Diet A				415+ for 3	10	7	0	0.0
II	Diet B	-51.3	-30.4	175.3 for 3		0	0	0	4.3
III	Diet B and 5% Petrol-Agar in diet	-40.3	-33.1	129 for 3		0	0	0	5.3
IV	Diet B and 5% mineral oil in diet	-54.3	-34.8	130.3 for 3		0	0	0	5.0
V	Diet B and mixture of 5% mineral oil and 1% cod liver oil in diet	-55.6	-22.3	288.3 for 3		1	0	4	3.3
VI	Diet B and mixture of 5% Petrol-Agar and 1% cod liver oil in diet	-37.6	-23.2	401.6 for 3		1	0	3	2.3

Incidence of disease, see page 31.

was 175 days. They were never pregnant.

Group III was put on diet B and 5 per cent Petrol-Agar mixed with the food. The life span in this group averaged 129 days, in comparison to 175 days for those on a straight A deficiency. There never were any signs of pregnancy. Two of the three had xerophthalmia, one associated with ulcerated corneas. Pro-stomach ulcers were present in all and in one case in association with very small craters in the antrum and in the other a few antrum lesions with marked fissuring as well as ulcers in the pro-stomach.

Group IV was fed diet B plus 5 per cent mineral oil mixed with the diet. The average length of life was 130 days and the weight loss of 54.3 gms., or 34.8 per cent, are similar to the results on the preceding group. All three of the group had gastric lesions. Two had several pro-stomach ulcers with bleeding bases and also erosions in the ventriculus. The third animal had four well-defined ulcers in the antrum near the pylorus and the pro-stomach lining was desquamated. Group V received a modified diet A, the modification consisting in 9 per cent butter and a mixture of 1 per cent cod-liver oil and 5 per cent mineral oil, mixed directly with the food. The average length of life for this group was 288.3 days, the last one dying on the 314th day. A loss of weight of 55.3 gms., or 22.3 per cent, was found. For the first six months of this experiment the average weight of this group ran very close to the controls but from then on a rapid falling off of the weight was observed. Necropsy revealed some slight gastric changes, i.e., one with a few antrum ulcers, a second with streaks of clotted blood in the main stomach and the third with a few bleeding points in the main stomach. The first one mentioned had had an intermittent hemorrhagic mucous colitis

which was severe at the time of death, but examination revealed only a slightly inflamed ileum. This rat had voided bloody urine for some time but no macroscopic kidney findings accounted for it and the bladder was only mildly inflamed. One pregnancy came to term with four dead young after the rat had twice showed signs of pregnancy which did not come to term. Another resorption occurred later. Only one case of xerophthalmia developed.

Group VI received the modified diet A and a mixture of 1 per cent cod-liver oil and 5 per cent Petrol-Agar. The body weight in this group was consistently below the controls and also below that of the preceding group. All were dead by the 405th day and the whole group lived an average of 401 days. Both revealed small ulcer craters in the antrum, and in one case they were well-defined. This rat had been suffering from a hemorrhagic colitis but except for a slight inflammation in the ileum showed no gross pathology. Also, she was the only one with xerophthalmia, which in her case was associated with a hemorrhagic exudate. The ♂ had one well-defined pro-stomach ulcer and pneumonia. One litter of three was born dead and there was no evidence of resorption.

Since there were some fatalities even among the controls and since the necropsy findings were variable, a scheme to present them in a comparative method was formulated. This assumes that the normal healthy rat has 0 illnesses and all major necropsy findings rate as 1. Among these findings impairment in growth, lesions in the pro-stomach, main stomach, or antrum, pneumonia, tuberculosis, tumors, xerophthalmia, severe diarrhea, marked alopecia, extreme emaciation, or death associated with pregnancy were considered each as 1. Thus each rat had a # from 0 up, depending upon the state of his health. Then these were averaged for each group and registered as "Incidence of Disease" on Tables

I, II, III, IV, V, VI, and VII. Finally the whole group of experiments was treated on a comparative basis. By this method it is shown (Table VIII) that a much higher incidence of disease results in the rats receiving mineral oil or Petrol-Agar and an A deficient diet than those receiving a straight A deficient diet. Those on the adequate diet receiving mineral oil or Petrol-Agar in the food or by tube nearly approach the straight A deficiency animals. The rats least affected are those on the cod-liver oil combinations or on recovered butter. These animals show a definite weight loss but less actual disease. However, all rats on mineral oil or Petrol-Agar seem to show a reduced resistance to infection, which is characteristic of A deficiency.

Experiment VIII

The intestinal rate work (Table IX) shows that the administration of 5 per cent mineral oil or Petrol-Agar definitely accelerates the intestinal rate. Sixty complete tests were run on eighteen rats. Only the complete tests were used, as a loss of beads might affect the initial or final rate. Twelve of these rats were distributed in the three following groups. Four controls (2 ♂s and 2 ♀s) were fed diet A, four other rats (2 ♂s and 2 ♀s) were fed diet A plus 5 per cent mineral oil mixed with the food, and four rats (2 ♂s and 2 ♀s) received diet A and 5 per cent Petrol-Agar mixed with their food. The four rats on diet A plus 5 per cent mineral oil mixed with the food showed an acceleration of 43 per cent in the initial, 6 per cent in the final, and 26 per cent in the average intestinal rate. The four rats on diet A and 5 per cent Petrol-Agar added to the diet showed an acceleration of 43 per cent in the initial, 23 per cent in the final, and 34

TABLE VIII. SUMMARY OF EFFECT OF MINERAL OILS
ON THE HEALTH OF 237 RATS

0 represents healthy rat.

1 represents any of the following symptoms or findings, i.e., impairment in growth, lesions in pro-stomach, ventriculus or antrum, pneumonia, tuberculosis, tumors, xerophthalmia, severe diarrhea, marked alopecia, extreme emaciation, death associated with pregnancy. Thus the higher the figure, the poorer the condition of the animal.

Diet	Number of animals	Incidence of disease
Controls on diet A	43	0.4
Controls on diet B	43	3.6
Diet B and mixture of 10% butter (vitamin A destroyed) 5% M.O.	5	5.0
Diet B and 5% P.A. by tube	7	4.0
Diet B and 5% P.A. mixed with food	10	4.0
Diet B and 5% M.O. mixed with diet	10	3.7
Diet A and 5% M.O. by tube	7	2.7
Diet A and 5% P.A. by tube	7	3.0
Diet A and 5% M.O. mixed with food	14	3.7
Diet A and 5% P.A. mixed with food	7	3.4
Diet B and mixture of 5% butter and 5% M.O.	7	3.6
Diet B and mixture of 10% butter and 5% M.O.	12	2.6
Diet B and mixture of 10% butter and 10% M.O.	7	2.9
Diet B and mixture of 15% butter and 10% M.O.	7	2.7
Diet B and 10% recovered butter by tube	12	2.8
Diet B and 10% recovered butter mixed with food	7	2.3
Diet B and supernatant by tube	5	2.8
Diet B and mixture of 1% C.L.O. and 5% M.O. by tube	7	2.5
Diet B and mixture of 2% C.L.O. and 5% M.O. by tube	7	2.7
Modified diet A and 1% C.L.O. and 5% M.O. in food	3	2.3
Modified diet A and 1% C.L.O. and 5% P.A. in food	3	2.3

Note:

Diet A = Adequate diet
Diet B = Diet deficient in
vitamin A

P.A. = Petrol-Agar
C.L.O. = Cod-liver oil
M.O. = Mineral oil

TABLE IX. INTESTINAL RATE MEASURED BY THE BEAD METHOD ON 18 RATS

	Mixed with food				Given by tube		
	Controls		Mineral oil		Petrol-Agar		Petrol-Agar
	♂s	♀s	♂s	♀s	♂s	♀s	
Initial	31.3	37.3	18.5	20.4	18.6	20.4	22.2
Final	66.8	82.1	78.5	61.9	68.8	45.8	48.2
Average	47.6	56.3	43.9	35.7	34.1	48.5	29.7

Note:

All rats were fed diet A, the adequate diet.

These figures represent the average of 60 complete tests on each of the two animals in each group, or 120 complete tests.

per cent in the average intestinal rate.

The remaining six animals (all females) were divided into three groups. Two controls were fed diet A, two rats were fed diet A plus 5 per cent mineral oil by tube, and two rats received diet A plus 5 per cent Petrol-Agar by tube. For the two rats fed diet A and 5 per cent mineral oil by tube the initial intestinal rate was accelerated 32 per cent, the final 10 per cent, and the average 13 per cent. The two rats fed diet A and 5 per cent Petrol-Agar by tube show an increase of initial intestinal rate of 32 per cent, of 40 per cent for the final, and of 34 per cent for the average. The final intestinal rates were influenced by the fact that there were several cases where a bead would fail to pass until a week had elapsed from giving the beads. This materially lengthened the final rates even when an average was taken and could only be counteracted by running a much longer series of animals than were used in this experiment.

Disoussion

The consistent results of impairment of growth revealed in all our experiments of rats on mineral oil administration of any kind differ from those of some of the other workers.

Rowntree fed 0.03 or 0.06 milligrams (1 or 2 drops) of cod-liver oil and 0.25 cc., 0.5 cc., or 0.75 cc. of mineral oil per day for 15 and 25 days mixed with a basal ration, and reported curative effects on rats previously deprived of vitamin A and growth comparable with control rats receiving no mineral oil. In our work, in Experiment VII, Group V, 0.825 cc. of a mixture of cod-liver oil and mineral oil containing 0.625 cc. (0.5 gm.) of mineral oil and 0.2 cc. (0.1 gm.) of cod-liver oil was fed with

an adequate diet. These rats had been thirty-five days on the control diet, not an A deficiency diet as in Rowntree's experiments. After this or upon the administration of the mixture, a continued gain in weight occurred for 104 days and then a decrease. If Rowntree had experimented on her rats for a longer time, growth might not have continued as favorably as after the first few days.

In another experiment Rowntree did not state what the source of vitamin was nor the exact amounts fed, but merely that enough was fed for maintenance to rats previously deprived of vitamin A. On 0.5 cc. of mineral oil, four of six animals died within thirty-one days. It is difficult to compare our results with those in the experiment of Rowntree. But considering that the rats in the Rowntree experiment were sixty to ninety days old at the beginning of the experiment and on an A deficiency diet approximately forty-two days and then none lived longer than thirty-one days after the mineral oil was added to the diet, their extreme age limit was 163 days. They lost an average weight of 50 gms. These results compare closely with our results on straight A starvation in rats of comparable age.

In some of Jackson's experiments 0.5 cc. of mineral oil was fed separately from 100 and 150 mgs. of butterfat to rats already depleted of vitamin A. In other experiments he compared the effect of feeding mineral oil mixed with 100 mgs. of butterfat and fed separately from the food with the same amount of mineral oil fed separately from the 100 mgs. of butterfat. No difference could be detected in the growth curves after being fed mineral oil separately for seventy-five days. In our work in Experiment I, Group III, when 1000 mgs. to 1500 mgs. of butterfat was fed mixed with the food and 0.55 to 0.87 cc. of mineral oil given separately by tube, a slightly lower average growth is shown. But

in Jackson's experiments it seems probable that they were run too short a time to bring out all the chronic effects of mineral oil. But he found that the growth curves of rats were depressed when the mineral oil and butterfat were mixed and fed apart from the food. Both Jackson and Rowntree concluded that an increase of vitamin A in the diet decreased the deleterious effects of mineral oil administration. To a certain extent this is true but in our work in Experiment II, Group V, where the rats are fed 3 cc. per day by tube of a mixture of butterfat and mineral oil containing 1.5 gms. of butter and 1 gm. of mineral oil separately from the A deficient diet B, the growth curve (Graph II) is lower than for the controls. Rats in Group III, in Experiment VI (Graph VI), which were fed 1.8 cc. of a mixture containing 1 gm. of butter and 0.5 gm. of mineral oil, showed a depression in the average weight (12%), which agrees with the above findings even when there is an excess of butter. In Experiment IV, Group VI (Graph IV), rats on the same regimen show a 23.5 per cent depression in average weight from their controls. These differences probably depend upon the different length control periods, i.e., 35 days in Experiment VI compared with 8 days in Experiment IV. In Experiment III, Groups V and VI (Graph III), where 5 per cent mineral oil and Petrol-Agar were mixed with the adequate diet a 26 per cent impairment in weight occurred in the former and a 17.6 per cent in the latter. In Experiment IV, Group V (Graph IV), where the rats were also fed 5 per cent mineral oil mixed with the adequate diet, a 23 per cent difference from the controls was observed.

In Burrow's experiments the large doses of 7 per cent to 20 per cent of mineral oil might account for the sudden deaths of his rats. But the diet might also be a factor. For in a preliminary experiment of ours, not described in our report, an attempt

was made to duplicate Burrow's work. In this study the controls died almost as rapidly as the experimental animals. From 24 rats, 6 on Burrow's control diet, 6 on his vitamin A deficient diet, 6 on his control diet plus 7 per cent mineral oil, and 6 on his control diet plus 14 per cent mineral oil, only one control and one 7 per cent mineral oil animal were alive after sixty days' observation.

The results of Dutcher, Ely and Honeywell are based on feeding such small doses of butter, i.e., 67 and 113 mgs. per day, to rats that signs of A deficiency are not surprising, because Jackson found that 100 mgs. of butter were necessary to cure A deficiency in rats.

In view of our results on experiments on intestinal rate, it is surprising that Olsen found no changes in either three dogs or six human subjects. It may be that the carmine method is not as reliable as the bead method or that the presence of hair in the dogs' feces served as roughage and masked the true effects of the mineral oil. For from the results (Table IX) the initial and average intestinal rates on 5 per cent mineral oil fed by tube to both ♂ and ♀ rats are accelerated compared with the controls receiving no mineral oil.

Conclusions

In feeding experiments lasting from 415 to 695 days, on 237 rats, results have been obtained that seem to warrant the following conclusions:

1. In all cases when mineral oil or Petrol-Agar in doses of 5 to 10 per cent of the diet was fed to rats an impairment in growth occurred compared with the controls receiving the same or

less vitamin A in butter or cod-liver oil.

2. Reproduction was impaired in all rats to whom mineral oil in the above quantities was being fed mixed with the food or separately. The effects varied from complete sterility to the inability to carry litters through lactation. In such cases the young were unable to live when separated from the mother, being small and undernourished compared with the young of control litter mate rats. Chronic deficiency in vitamin E (due to absorption in the mineral oil) may be a factor in these effects.

3. Rats on a vitamin A deficient diet plus 5 per cent mineral oil or Petrol-Agar show a shorter life span, greater loss of body weight, and higher incidence of disease (especially gastrointestinal) than rats on the straight vitamin A deficient diet. Whether this is due to chronic poisoning from some toxic substance in the mineral oil or to an interference with digestion and absorption of food could not be determined.

4. The average rate of passage of beads through the gastrointestinal tract of rats who were being fed 5 per cent mineral oil mixed with the adequate diet was accelerated 8 per cent in the males and 37 per cent in the females. The average intestinal rate for female rats on 5 per cent Petrol-Agar mixed directly with the adequate diet was accelerated 35.5 per cent. For male rats on the same regimen as above, the gastro-intestinal motility was increased 28 per cent. Female rats who were fed an adequate diet and 5 per cent Petrol-Agar by tube showed a 39 per cent acceleration of their average intestinal rate. The average intestinal rate for female rats fed 5 per cent mineral oil by tube plus an adequate diet was accelerated 13 per cent.

5. In man the therapeutic use of mineral oil or Petrol-Agar is usually not continuous although often taken over a long

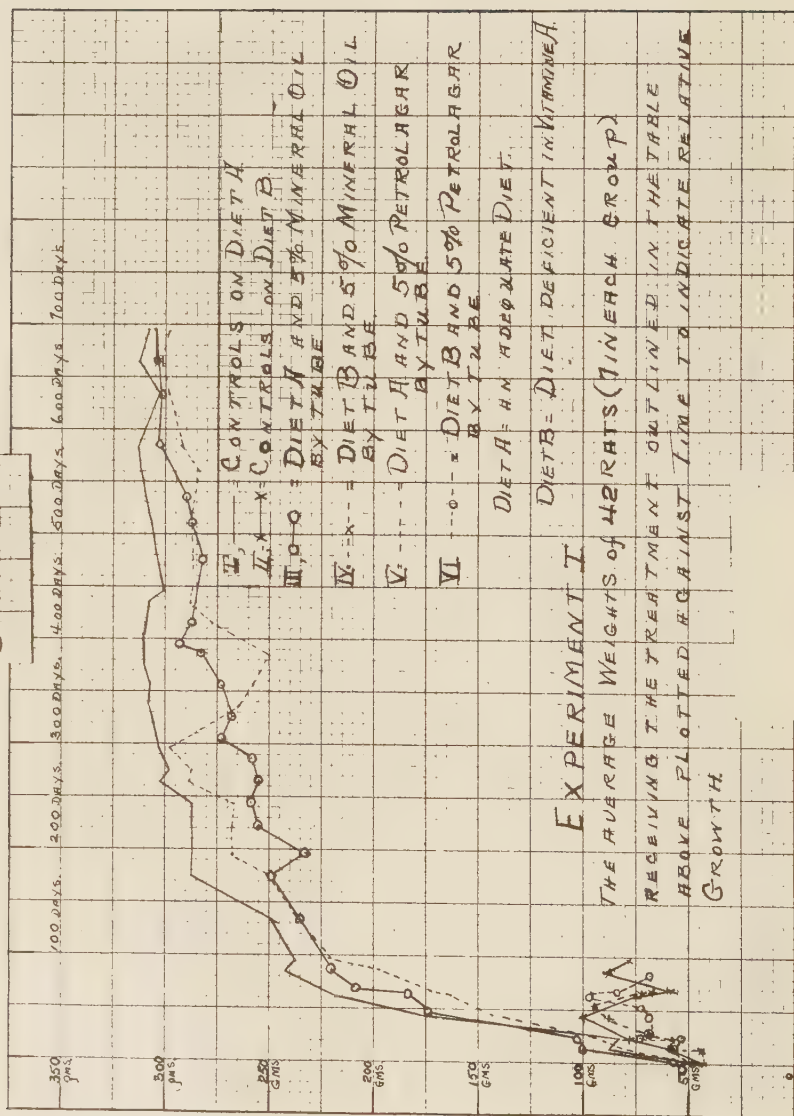
period. The average individual probably, also, uses a diet higher in fat-soluble vitamins than that used in our experimental diets. Moreover, the therapeutic administration of mineral oil or Petrol-Agar is not very common in childhood or early youth. In contrast to this, in our experiments rats were fed mineral oil or Petrol-Agar in doses of 5 to 10 per cent of the diet for periods beginning at an early age and continuing for from one-half to four-fifths of the normal life span of the rats.

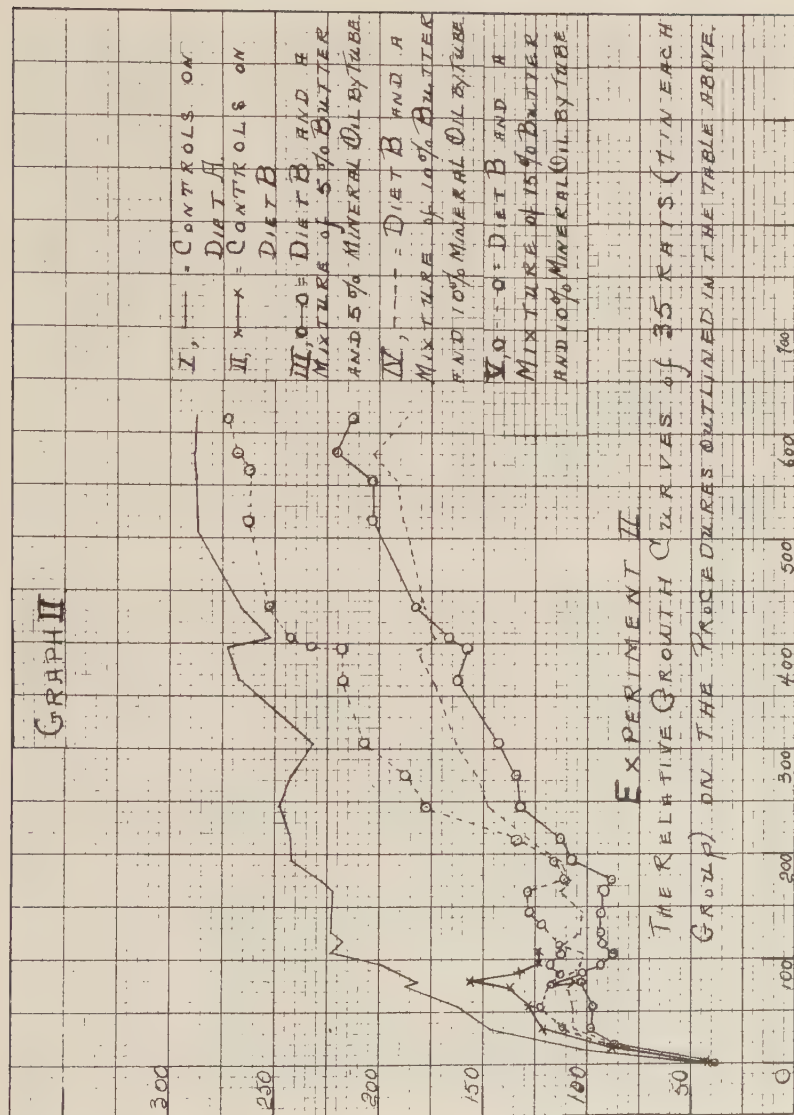
The above facts may account for the apparent absence in clinical practice of the effects observed in this study.

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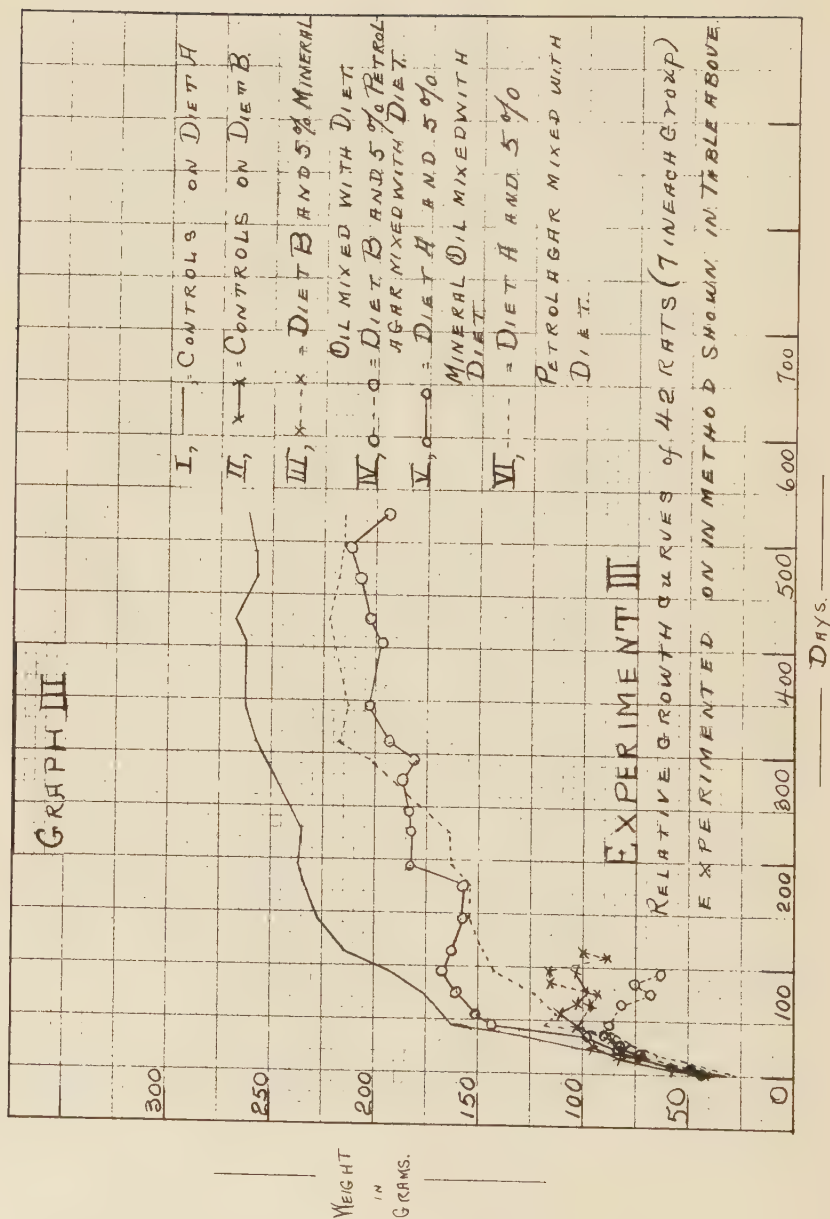
GRAPH I





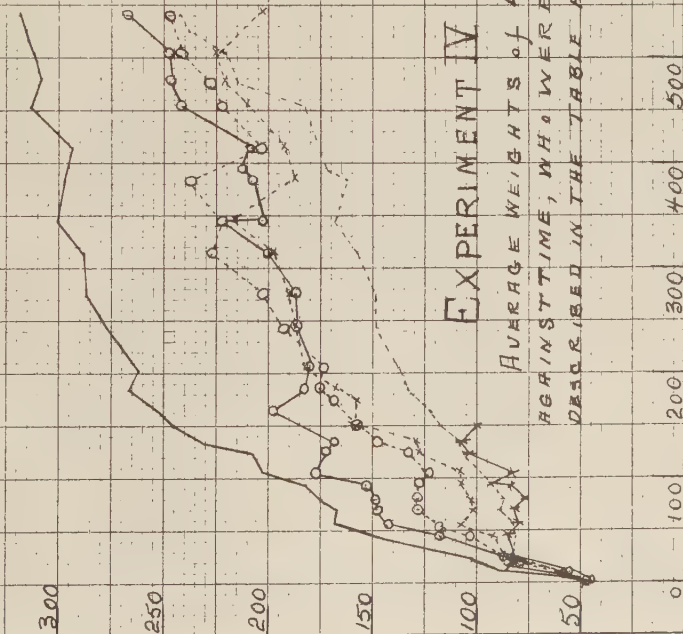
WEIGHT
IN
GMS.

Days.



GRAPH IV

- I, —, CONTROLS ON DIET A.
- II, x-x=CONTROLS ON DIET B.
- III, o-o=DIET B AND 10% RECOVERED BUTTER MIXED WITH FOOD.
- IV, x-x=DIET B AND 10% RECOVERED BUTTER BY TUBE.
- V, o-o=DIET B AND 5% MINERAL OIL MIXED WITH FOOD.
- VI, —, DIET B AND MIXTURE OF 10% BUTTER AND 5% MINERAL OIL BY TUBE.

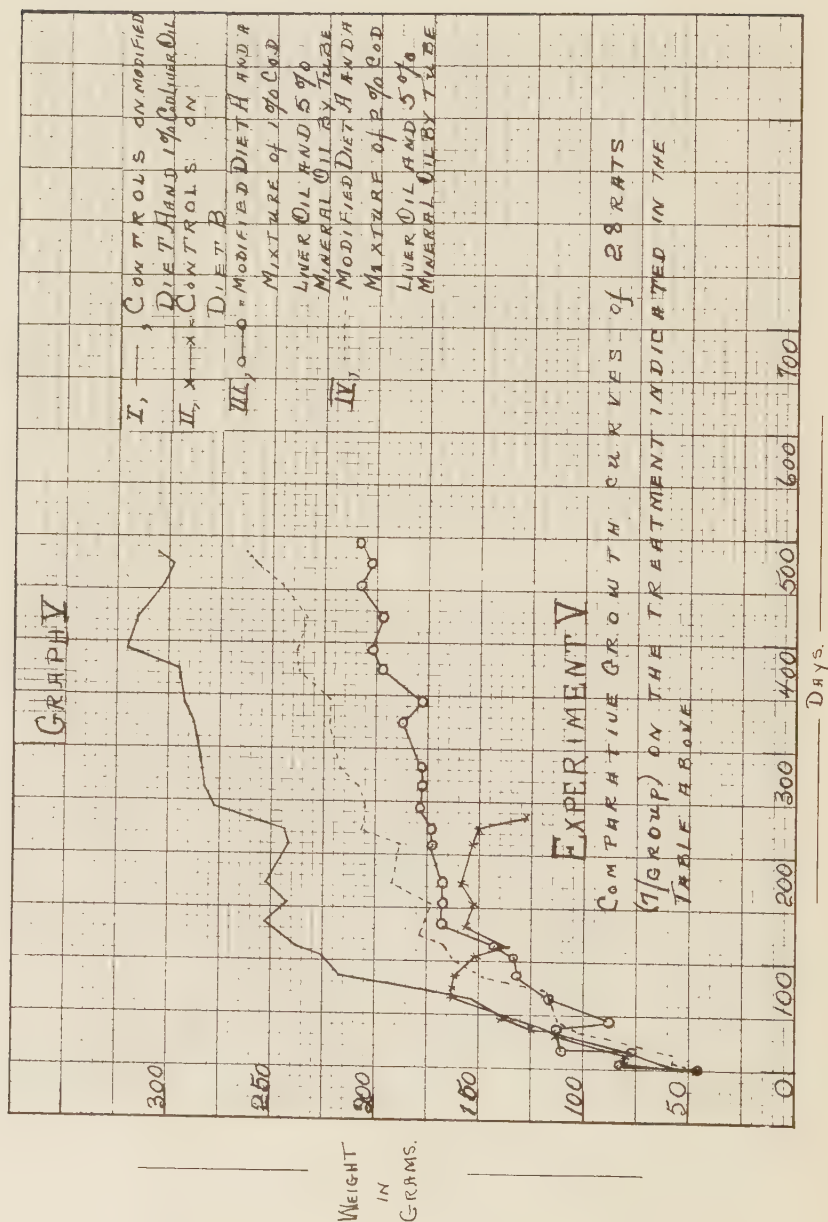


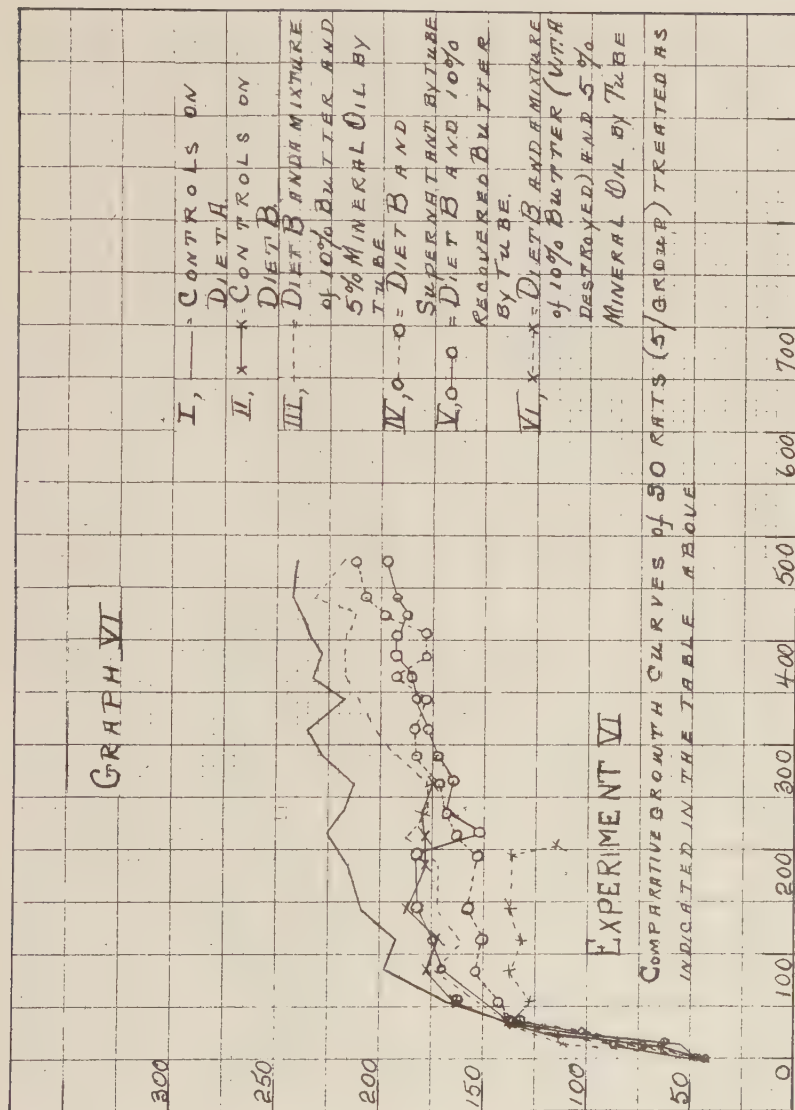
EXPERIMENT IV

AVERAGE WEIGHTS OF 42 RATS (7 GROUP) PLOTTED AGAINST TIME, WHO WERE ON THE REGIMEN DESCRIBED IN THE TABLE ABOVE.

WEIGHT
IN
GRAMS

DAYS





WEIGHT
IN
GRAMS

DAYS

